EOD records of its three closest relatives[3] (Fig. 4b). The EOD of the monophasic *Brachyhypopomus* species is similar to that of the electric eel in waveform, duration and spectrum[24] (Fig. 4a). The resting discharge rate was low (8–13 Hz), resembling an alert electric eel in both rate and variability[24]. Calibrated daytime EOD amplitudes were 1.5–1.8 mV cm^{-1} at 10 cm, which is between five and ten times greater than specimens of its sister species[3] *Brachyhypopomus* sp. 2 (Fig. 4b). Thus the monophasic *Brachyhypopomus* species appears to have lost the second phase of its EOD and boosted EOD amplitude, consistent with Hagedorn's proposal that this fish is a batesian electric mimic of the sympatric electric eel. Further confirmation of the hypothesis could come only from experiments showing mutual avoidance of electric eels and monophasic *Brachyhypopomus* by an electroreceptive predator.

In summary, predation avoidance is the strongest candidate as the driving force for the initial evolution of EOD complexity, in particular, the transition from primitive monophasy to biphasy. This conclusion is supported by three lines of evidence: (1) spectral comparison of monophasic and biphasic EODs; (2) demonstration that biphasic pulses are less detectable by a known electroreceptive predator; and (3) examples of specific adaptations (high voltage, geographic isolation and mimicry) that protect species with monophasic EODs. Sexual dimorphism in the second EOD phase of *Brachyhypopomus* spp. (Fig. 2) seems to be the secondary modification of an adaptation for signal crypsis. Evolutionary escape from predation has been cited as a key factor promoting adaptive radiation[25]. Thus spectral shifting may have contributed to the success of this order in tropical South America. A key question is whether signal multiphasy evolved in any gymnotiforms outside the geographic range of their electroreceptive predators. Several extant multiphasic gymnotiform taxa extend beyond the range of large electroreceptive predators[23] (O. Macadar, personal communication), but their centres of distribution lie in the predator-rich continental tropics and none could be argued to represent an independent origin of multiphasy.

A parallel story may emerge from Africa, where mormyrid electric fish have undergone extensive radiation and an electroreceptive predator, the catfish *Clarias,* serves as their major predator[26,27]. Nor are electric fish entirely unique in having protective signal adaptations exploited by sexual selection. Ctenuchid moths evolved acoustic signals to alert predatory bats of their toxicity, and these signals have likewise been co-opted for mate attraction[28,29]. □

Methods

An electric eel 1 m long was trained to receive food (goldfish) when it approached any playback of an electric field in its round aquarium (120 cm diameter, 60 cm deep). We played electric stimuli from a DC-coupled 5-cm carbon dipole at calibrated intensities equivalent to natural EODs[4]. DC offset at 10 cm from the dipole centre was less than 0.05 μV cm^{-1}. Training stimuli included a wide variety of monophasic and biphasic digitized EODs. Experimental stimuli included the biphasic EOD of a female *Brachyhypopomus pinnicaudatus* and the same EOD with the second phase digitally removed (Fig. 3c, d). Trials were sequenced randomly. Playbacks of 1 min duration began while the eel rested on the tank bottom, at a distance of more than 60 cm from the electrode. A 'blind' assistant rewarded all electrotactic approaches with food. In the first set of trials, playback rate simulated a repeated social signal, 0.5 s at 50 Hz alternated with 0.5 s at 200 Hz. In a second set of trials playing the truncated stimulus only, rate modulation had no effect on frequency of approach (19/21 trials with rate modulation compared with 19/20 trials at 50 Hz). I measured EOD amplitudes (Fig. 4b) by methods published previously[4].

Received 30 March; accepted 19 May 1999.

1. Darwin, C. *The Descent of Man, and Selection in Relation to Sex* (Murray, London, 1871).
2. Andersson, M. *Sexual Selection* (Princeton Univ. Press, 1994).
3. Sullivan, J. P. *A Phylogenetic Study of the Neotropical Hypopomid Electric Fishes (Gymnotiformes: Rhamphichthyoidea)*. Thesis, Duke University (1997).
4. Franchina, C. R. & Stoddard, P. K. Plasticity of the electric organ discharge waveform of the electric fish *Brachyhypopomus pinnicaudatus*: I. Quantification of day-night changes. *J. Comp. Physiol. A* **183,** 759–768 (1998).
5. Lissmann, H. W. On the function and evolution of electric organs in fish. *J. Exp. Biol.* **35,** 156–191 (1958).
6. Bullock, T. Electroreception. *Annu. Rev. Neurosci.* **5,** 121–170 (1982).
7. Bennett, M. L. V. Modes of operation of electric organs. *Ann. NY Acad. Sci.* **94,** 458–509 (1961).
8. Kirschbaum, F. in *Electric Fishes History and Behavior* (ed. Moller, P.) 267–301 (Chapman & Hall, London, 1995).
9. Bennett, M. L. V. in *Fish Physiology* (eds Hoar, W. S. & Randal, D. J.) 493–574 (Academic, New York, 1971).
10. Szabo, T. & Fessard, A. in *Handbook of Sensory Physiology* (ed. Fessard, A.) 59–124 (Springer, Berlin, 1974).
11. Hagedorn, M. Ecology and behavior of a pulse type electric fish, *Hypopomus occidentalis* (Gymnotiformes, Hypopomidae), in a fresh water stream in Panama. *Copeia* **1988,** 324–335 (1988).
12. Westby, G. W. M. The ecology, discharge diversity and predatory behaviour of gymnotiforme electric fish in the coastal streams of French Guiana. *Behav. Ecol. Sociobiol.* **22,** 341–354 (1988).
13. Hopkins, C. D., Comfort, N. C., Bastian, J. & Bass, A. H. Functional analysis of sexual dimorphism in an electric fish, *Hypopomus pinnicaudatus*, order Gymnotiformes. *Brain Behav. Evol.* **35,** 350–367 (1990).
14. Shumway, C. A. & Zelick, R. D. Sex recognition and neuronal coding of electric organ discharge waveform in the pulse-type weakly electric fish, *Hypopomus occidentalis*. *J. Comp. Physiol. A* **163,** 465–478 (1988).
15. Reid, S. La biologia de los bagres rayados *Pseudoplatystoma fasciatum* y *P. tigrinum* en la cuenca del rio Apure – Venezuela. *Revista UNELLEZ Ciencia Tecnol.* **1,** 13–41 (1983).
16. Dunning, B. B. *A quantitative and comparative analysis of the tonic electroreceptors of* Gnathonemus, Gymnotus *and* Kroptypterus. Thesis, University of Minnesota (1973).
17. McCreery, D. B. Two types of electroreceptive lateral lemniscal neurons of the lateral line lobe of the catfish *Ictalurus nebulosus*; connections from the lateral line nerve and steady-state frequency response characteristics. *J. Comp. Physiol.* **113,** 317–339 (1977).
18. Peters, R. C. & Buwalda, R. J. A. Frequency response of the electroreceptors ("small pit organs") of the catfish, *Ictalurus nebulosus* LeS. *J. Comp. Physiol.* **79,** 29–38 (1972).
19. Hopkins, C. D. & Heiligenberg, W. Evolutionary designs for electric signals and electroreceptors in gymnotoid fishes of Surinam. *Behav. Ecol. Sociobiol.* **3,** 113–134 (1978).
20. Hopkins, C. D. Stimulus filtering and electroreception: tuberous electroreceptors in three species of gymnotiform fish. *J. Comp. Physiol.* **111,** 171–208 (1976).
21. Moller, P. *Electric Fishes History and Behavior* (Chapman & Hall, London, 1995).
22. Alves-Gomes, J. A., Orti, G., Haygood, M., Heiligenberg, W. & Meyer, A. Phylogenetic analysis of the South American electric fishes (order Gymnotiformes) and the evolution of their electrogenic system: a synthesis based on morphology, electrophysiology, and mitochondrial sequence data. *Mol. Biol. Evol.* **12,** 298–318 (1995).
23. Miller, R. R. Geographic distribution of Central American freshwater fishes. *Copeia* **1966,** 773–802 (1966).
24. Crampton, W. G. R. Electric signal design and habitat preferences in a species rich assemblage of gymnotiform fishes from the upper Amazon basin. *An. Acad. Bras. Ci.* **70,** 805–847 (1998).
25. Ehrlich, P. R. & Raven, P. H. Butterflies and plants: a study in coevolution. *Evolution* **18,** 586–608 (1964).
26. Merron, G. S. Pack-hunting in two species of catfish, *Clarias gariepinus* and *C. ngamensis*, in the Okavango Delta, Botswana. *J. Fish Biol.* **43,** 575–584 (1993).
27. Hopkins, C. D. in *Electroreception* (eds Bullock, T. H. & Heiligenberg, W.) 527–576 (Wiley, New York, 1986).
28. Sanderford, M. V. & Conner, W. E. Acoustic courtship communication in *Syntomeida epilais* Wlk. (Lepidoptera: Arctiidae, Ctenuchinae). *J. Insect Behav.* **8,** 19–32 (1995).
29. Simmons, R. B. & Conner, W. E. Ultrasonic signals in the defense and courtship of *Euchaetes egle* Drury and *E. bolteri* Stretch (Lepidoptera: Arctiidae). *J. Insect Behav.* **9,** 909–919 (1996).
30. Albert, J. S. & Fink, W. L. *Sternopygus xingu*, a new species of electric fish from Brazil (Teleostei: Gymnotoidei), with comments on the phylogenetic position of *Sternopygus*. *Copeia* **1996,** 85–102 (1996).

Acknowledgements. M. Olman trained and tested the electric eel, which was provided by H. Zakon. T. Lopez donated its food. F. Torres provided me with live gymnotiforms from Peru, photographed by G. Valcarce. The Organization for Tropical Studies supported field collections with B. Rasnow. J. Alves-Gomes, W. Crampton, W. Heiligenberg, C. Hopkins, J. Sullivan, D. Taphorn and particularly M. Hagedorn contributed helpful insights. T. Collins, K. Condon, G. Read, M. Ryan and J. Trexler improved the manuscript. Support came from the NIH. Experiments complied with NIH 'Principles of Animal Care' No. 86-23, rev. 1985.

Correspondence and requests for materials should be addressed to P.K.S. (e-mail: stoddard@fiu.edu).

'Green revolution' genes encode mutant gibberellin response modulators

Jinrong Peng*, Donald E. Richards*, Nigel M. Hartley, George P. Murphy, Katrien M. Devos, John E. Flintham, James Beales, Leslie J. Fish, Anthony J. Worland, Fatima Pelica, Duraialagaraja Sudhakar†, Paul Christou, John W. Snape, Michael D. Gale & Nicholas P. Harberd

John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK
** These authors contributed equally to this work*

World wheat grain yields increased substantially in the 1960s and 1970s because farmers rapidly adopted the new varieties and cultivation methods of the so-called 'green revolution'[1–4]. The new varieties are shorter, increase grain yield at the expense of straw biomass, and are more resistant to damage by wind and

† Present address: Rice Transformation Laboratory, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore-641 003, India.

© 1999 Macmillan Magazines Ltd

rain[3,4]. These wheats are short because they respond abnormally to the plant growth hormone gibberellin. This reduced response to gibberellin is conferred by mutant dwarfing alleles at one of two *Reduced height-1* (*Rht-B1* and *Rht-D1*) loci[4,5]. Here we show that *Rht-B1/Rht-D1* and maize *dwarf-8* (*d8*)[6,7] are orthologues of the *Arabidopsis Gibberellin Insensitive* (*GAI*) gene[8,9]. These genes encode proteins that resemble nuclear transcription factors and contain an SH2-like[10] domain, indicating that phosphotyrosine may participate in gibberellin signalling. Six different orthologous dwarfing mutant alleles encode proteins that are altered in a conserved amino-terminal gibberellin signalling domain. Transgenic rice plants containing a mutant *GAI* allele give reduced responses to gibberellin and are dwarfed, indicating that mutant *GAI* orthologues could be used to increase yield in a wide range of crop species.

Gibberellin is an essential endogenous regulator of plant growth[11]. *Rht-B1b* and *Rht-D1b* are semidominant, altered function (rather than loss-of-function) mutant alleles of the *Rht-1* height-regulating genes of wheat. These mutant alleles reduce plant height (Fig. 1a), reduce responses to gibberellin and increase *in planta* gibberellin levels[4,5,12,13]. These properties are also characteristic of mutant alleles of maize *d8*[6,7,14] and of the *Arabidopsis gai* allele[8,9,15], indicating that these mutant alleles might define orthologous genes that are involved in gibberellin signalling. *GAI* (the wild-type allele) encodes a protein (GAI) containing features that are characteristic of transcription factors[9]. The *gai* allele encodes a mutant protein (gai), lacking 17 amino acids from near the amino terminus, that is thought to confer the altered gibberellin responses characteristic of the *gai* mutant[9]. Database searches revealed a rice expressed-sequence tag (EST; D39460) that encodes a potential polypeptide containing a sequence nearly identical to these 17 amino acids[16]. We used this EST to investigate whether the dominant dwarfing mutant alleles of *GAI*, *Rht-1* and *d8* identify orthologous genes in *Arabidopsis*, wheat and maize.

D39460 was used to isolate wheat complementary DNA C15-1. The genome of bread wheat is hexaploid, consisting of three homoeologous chromosome sets (the A, B and D genomes). Analysis of lines lacking particular chromosomes (nullisomic) showed that C15-1 hybridized to genomic DNA fragments derived from wheat chromosomes 4A, 4B and 4D (Fig. 1b), correlating with the location of the *Rht-1* alleles (all known mutant *Rht-1* alleles are on chromosomes 4B or 4D; ref. 5). Furthermore, restriction-

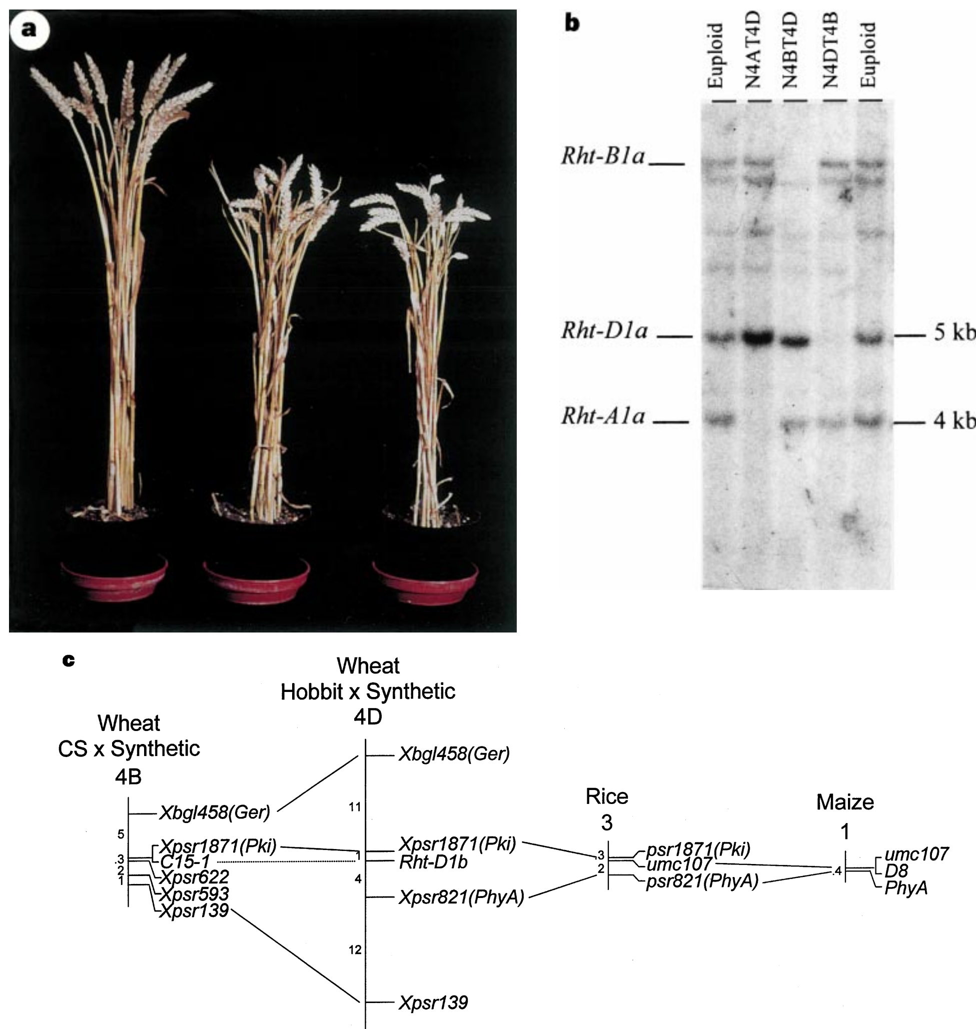
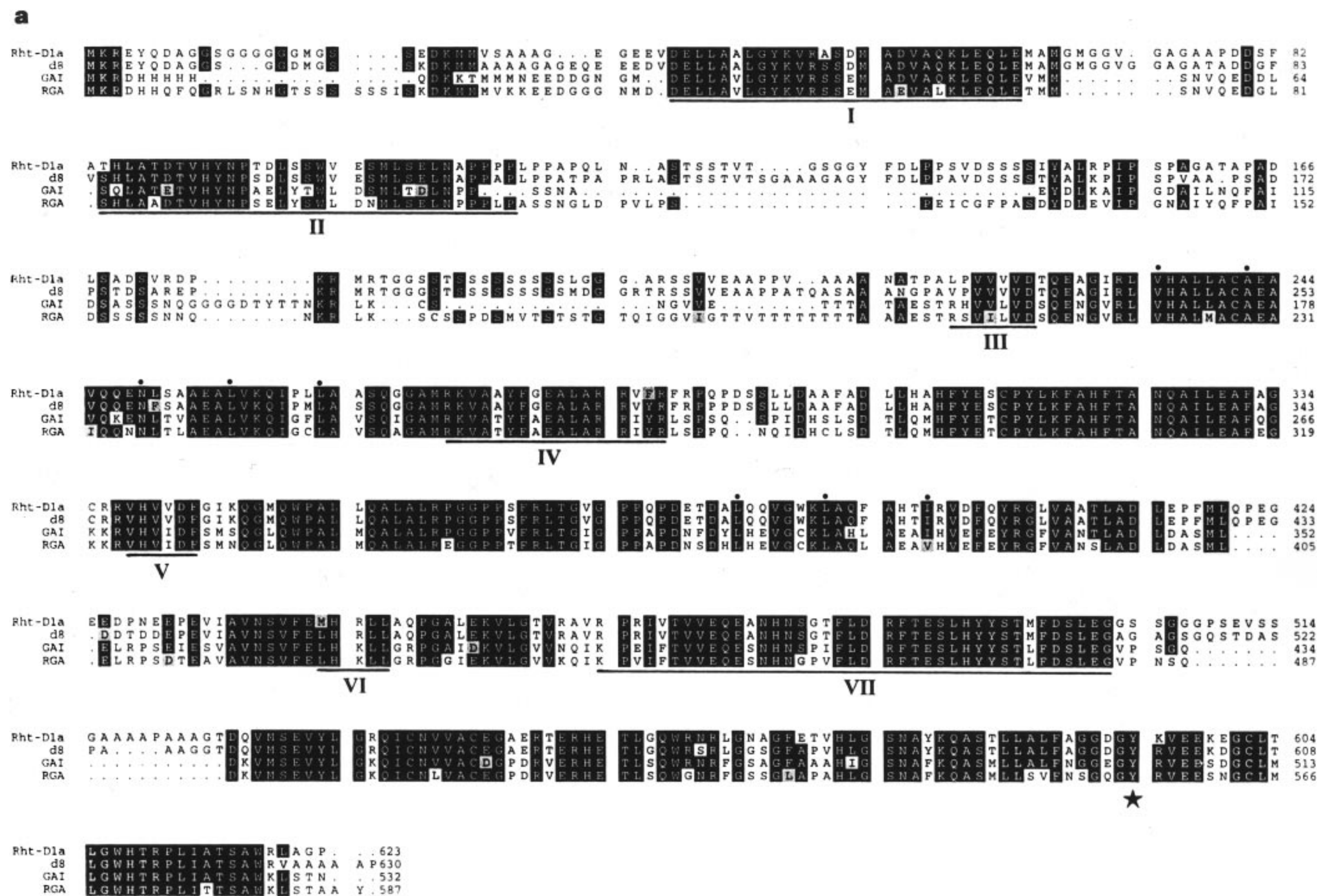


Figure 1 cDNA C15-1 maps to the *Rht-1* locus. **a**, Near-isogenic dwarf wheat lines: left, tall control (var. Mercia); centre, semi-dwarf *Rht-B1b*; right, semi-dwarf *Rht-D1b*. **b**, Gel-blot hybridization of C15-1 with *Dra1*-digested DNA from wheat lines lacking individual group 4 chromosomes (nullisomic 4A-tetrasomic 4D, N4AT4D; nullisomic 4B-tetrasomic 4D, N4BT4D; nullisomic 4D-tetrasomic 4B, N4DT4B), and euploid control (all var. Chinese Spring). Hybridizing fragments were assigned to chromosomes (4A, 4B and 4D) as shown. **c**, Partial linkage maps of wheat chromosomes 4B (ref. 26) and 4D, rice chromosome 3 (ref. 28), and maize chromosome 1 (ref. 29) showing the colinearity between regions containing C15-1, *Rht-D1b* and *D8-1*. A putative maize *d8* genomic fragment (see text) also displayed tight linkage with *umc107* (not shown). Wheat 4B data are from the F_2 of a Chinese Spring (CS) × Synthetic cross. Wheat 4D data are from the F_2 of a Hobbit (contains *Rht-D1b*) × Synthetic cross; segregation for *Rht-D1b* was assayed by seedling responses to gibberellin[12]. Map distances are in centiMorgans (cM).

© 1999 Macmillan Magazines Ltd

fragment length polymorphism mapping showed that C15-1 is tightly linked to *Xpsr1871(Pki)*, a marker that is itself tightly linked with *Rht-D1b* (Fig. 1c). Cereal genomes show substantial conservation in gene order (colinearity). The region of wheat chromosomes 4A, 4B and 4D to which C15-1 hybridizes is colinear with a segment of rice chromosome 3, and with a segment of maize chromosome 1 containing *d8* (Fig. 1c)[17]. These observations are consistent with the hypothesis that the C15-1 transcript is derived from one of the *Rht-1* homoeoalleles.

We used C15-1 to isolate genomic DNA clones containing the putative *Rht-B1a* and *Rht-D1a* (the *Rht-B1* and *Rht-D1* wild-type alleles[5]) and maize *d8* (the *D8* wild-type allele[7]) genes. The amino-acid sequences of the proteins encoded by wheat *Rht-D1a* (Rht-D1a), maize *d8* (d8) and *Arabidopsis GAI* (GAI) and *RGA* (RGA) were compared (Fig. 2a; RGA is an *Arabidopsis* gibberellin signalling protein that is closely related to GAI; ref. 16). Rht-D1a and d8 appear to be more closely related to GAI than they are to RGA (per cent amino-acid identity, GAI versus RGA is, respectively, 62 versus 58% (Rht-D1a) and 62 versus 59% (d8)). The carboxy-terminal ~2/3 of all four proteins are very similar to each other and to the equivalent region of SCARECROW (SCR), a candidate transcription factor from *Arabidopsis*[9,16,18], and of LATERAL SUPPRESSOR (Ls), a tomato protein required for formation of axillary branches during vegetative growth[19]. The N-terminal regions of the GAI/RGA/Rht-D1a/d8 proteins contain two regions of closely related sequence (regions I and II in Fig. 2a). Regions I and II are found in five gibberellin signalling proteins (GAI, RGA, Rht-D1a, d8 and also in Rht-B1a, the *Rht-B1a* gene product; Fig. 3a), but are not found in SCR or Ls, indicating that they may be responsible for the gibberellin-specific action of these proteins[9,16]. Furthermore,



b

```
             ◆             * ◆ ◆
Rht-D1a   R—(15)—GTFLDRFTES —(0)—LHYY— (3)—FDSLEGG
c-Abl     R—(12)—GSFLVRESES—(15)—YHYR—(16)—FNTLAEL
c-Src     R—(14)—GTFLVRESET—(20)—KHYK—(16)—FSSLQQL
PLC-γ1N   R—(21)—GSFLVRESET—(15)—QHCR—(14)—FDSLYDL
Dd-Stat   R—(12)—GTFIIRFSER—(18)—KHYL—(16)—FLSEHSQ
Stat5a    K—(12)—GTFLLRFSDS —(2)—GGIT— (4)—FDSPERN
```

Figure 2 Structural features of Rht-D1a, d8, GAI and RGA. **a**, Amino-acid sequence alignment comparing Rht-D1a, d8, GAI[9] and RGA[16]. Gaps are introduced to maximize alignment. Exact matches are boxed in black, shaded boxes indicate conservative substitutions. Indicated regions of conserved amino-acid sequence are: I and II, N-terminal regions that are not present in SCR[18] or Ls[19]; III and V, valine-rich regions; IV, nuclear-localization signal[9,16]; VI, LXXLL motif[9,16]; VII, SH2-like domain. Residues defining leucine heptad repeats are indicated by closed circles. Star, potential site of tyrosine phosphorylation (this feature, leucine heptad repeats, regions III, V and VII, and the relative positions of all of these features/regions are characteristic of STATs; ref. 22). **b**, Amino-acid sequence alignment comparing sequence from region VII of Rht-D1a (residues 464–503) with those of previously recognized SH2 domains. Functionally significant residues are in bold and italicized. Numbers in parentheses refer to the number of intervening residues that are not shown. A typical SH2 domain is a peptide stretch of 100 amino acids containing an invariant arginine (***R*** with asterisk) that recognizes the phosphate group of phosphotyrosine. Following this arginine are other strongly conserved residues (diamonds). These residues, together with another upstream arginine or lysine (***R*** or ***K*** with diamond), interact with the phosphate group, the tyrosine ring, and the polypeptide backbone of the ligand[20,21]. Other regions of the SH2 domain are less conserved, although their three-dimensional structures are similar. Sequences shown are c-Abl[10], c-Src[10], PLC-γ1N[10], Dd-Stat[30] and Stat5a[21].

© 1999 Macmillan Magazines Ltd

region I is substantially deleted in the *Arabidopsis gai* mutant, confirming that this region is important for gibberellin signalling[9].

Analysis of the GAI/RGA/Rht-D1a/d8 sequences revealed an SH2-like domain within the C-terminal section of the protein (region VII in Fig. 2; Fig. 2b). SH2 domains are associated with phosphotyrosine signalling in metazoans[10], and bind tyrosine-phosphorylated polypeptides at an essential arginine residue. This residue is invariant in SH2 domains and is found in the GAI/RGA/Rht-D1a/d8 SH2-like domain (Fig. 2a, b). Alignment of the Rht-D1a SH2-like domain with previously identified SH2 domains reveals substantial conservation of the amino-acid sequence, especially of those residues that assist in the binding of the phosphorylated tyrosine to the invariant arginine[20,21] (Fig. 2b). To our knowledge, this is the first identified putative SH2 domain in plants. STAT (signal transducers and activators of transcription) proteins are transcriptional regulators that contain SH2 domains[22]. GAI/RGA/Rht-D1a/d8 are candidate transcription factors that contain an SH2-like domain, and display other features characteristic of STATs (for details, see Fig. 2a). Phosphotyrosine signalling may be involved in gibberellin-mediated plant growth regulation, using proteins similar to the STAT factors that mediate cytokine/growth-factor control of growth in animals.

Rht-B1, *Rht-D1* and *d8* are defined by allelic series of semi-dominant mutations that confer differing severities of dwarfism[4–7]. To identify the molecular basis of these mutations, the DNA sequences of five mutant alleles (*Rht-B1b*, *Rht-D1b* and three *D8* alleles) were determined. Each allele contains a mutation that alters the N-terminal region of the protein that it encodes (Fig. 3a). All three maize mutant proteins (D8-1, D8-2023 and D8-Mpl), like *Arabidopsis* gai, lack regions of the peptide sequence. *D8-1* and *D8-2023* are, like *gai*, in-frame deletion mutations. In D8-1, D55 is replaced by a glycine, and 56-VAQK-59 are missing. This segment is very close to that deleted in gai, and falls within the highly conserved region I (Fig. 3a). D8-2023 lacks 87-LATDTVHYNPSD-98 from within the highly conserved region II (Fig. 3a). The *D8-Mpl* mutation is a 330-base pair (bp) deletion that extends from the 5′ untranslated sequence through the presumed (normal) start ATG codon and ends at V84. Genetic analysis[6] indicates that *D8-Mpl*, like *D8-1*, makes an active product. Presumably, *D8-Mpl* translation initiates at M106 (or a subsequent methionine), and makes an N-terminally truncated product that lacks region I and most of region II (Fig. 3a).

The *Rht-B1b* and *Rht-D1b* mutations are both nucleotide substitutions that create stop codons. In *Rht-B1b*, a T-for-C substitution converts the Q64 codon (CGA) to a translational stop codon (TGA; Fig. 3a). In *Rht-D1b*, a T-for-G substitution converts the E61 codon (GGA) to a translational stop codon (TGA; Fig. 3a). The similarity of the *Rht-B1b* and *Rht-D1b* mutations presumably explains why they confer very similar severities of dwarfism[4]. Genetic analysis indicates that both *Rht-B1b* and *Rht-D1b* make active products[12]. It is possible that the short N-terminal peptide fragments encoded by *Rht-B1b* and *Rht-D1b* confer the mutant phenotype. However, it is also possible that ribosomal scanning following translational termination at the mutant stop codons in *Rht-B1b* and *Rht-D1b* permits translational reinitiation at one or other of the several methionines that closely follow these stop codons[23], and that the resultant N-terminally truncated product confers the mutant phenotype. This seems more likely, as the *D8-Mpl* allele also encodes an N-terminally truncated product (see above). Thus *Rht-B1b* and *Rht-D1b*, like *D8-Mpl*, apparently encode N-terminally truncated products that lack region I (Fig. 3a).

Mutagenesis of *Arabidopsis gai* can generate apparent loss-of-function derivative alleles which confer a tall, rather than dwarf, phenotype[24]. These derivative alleles carry mutations that interrupt the *gai* open reading frame (ORF)[9] and thereby abolish *gai* function. Similarly, following fast-neutron mutagenesis, we obtained an apparent loss-of-function allele derived from wheat *Rht-B1b*. This new allele (*Rht-B1g*) confers a tall, gibberellin-responsive phenotype, rather than the dwarf, gibberellin-resistant phenotype characteristic of its *Rht-B1b* progenitor. Gel-blot analysis showed that *Rht-B1g* lacks C15-1-hybridizing DNA derived from chromosome 4B (the chromosome that carries *Rht-B1b*; Fig. 3b), indicating that *Rht-B1g* is a deletion mutation that abolishes *Rht-B1b* function.

The demonstration that, for multiple independent mutant alleles, a heritable change in phenotype is associated with a mutation in a candidate gene is conventionally used as proof that the candidate gene is indeed responsible for the phenotype being studied. Here we have shown that, for three independent mutant *d8* alleles, a heritable change in phenotype (dwarfism, reduced gibberellin response) is associated with a mutation in a candidate *GAI*-like gene. This shows

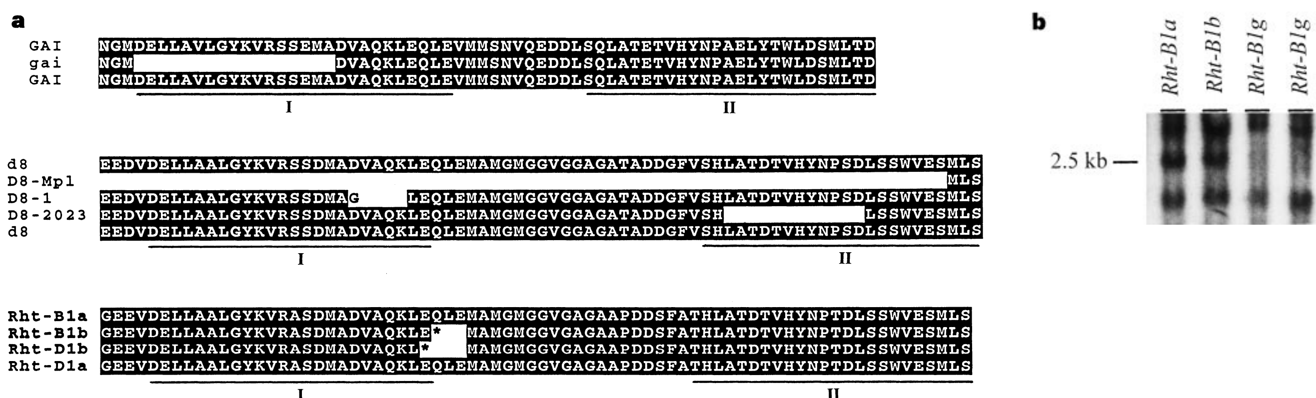


Figure 3 Dominant mutant alleles encode proteins with mutant N termini. **a**, N-terminal segments of predicted proteins encoded by mutant alleles *gai*, *D8-1*, *D8-2023*, *D8-Mpl*, *Rht-B1b* and *Rht-D1b* are compared with those of their respective wild-type alleles (*GAI*, *d8*, *Rht-B1a* and *Rht-D1a*). For each locus, the wild-type sequence is shown above and below the mutant sequence(s). Differences between wild-type and mutant sequences (deletions and substitutions) are highlighted in white, the position of translational stop codons is represented by an asterisk, and the previously identified highly conserved regions I and II (Fig. 2a) are shown. All mutations alter the N-terminal region of their encoded proteins, and affect regions I and/or II. D8-2023 also carries a 6-bp deletion that removes one G and one A residue from 510GAGA513, and a nucleotide substitution that converts T519 to A519 (with respect to d8 sequence; Fig. 2a, and data not shown). Because these altered residues are poorly conserved in GAI/RGA/Rht-D1a/d8, these changes are considered not to be of phenotypic significance. In wheat, Q64 of Rht-B1a is equivalent to Q62 of Rht-D1a, owing to a difference of two amino-acid residues in a poorly conserved N-terminal region (see text; data not shown). *gai* was isolated following X-irradiation mutagenesis[8]; all other mutant alleles shown are of spontaneous origin[4,6,7]. **b**, Gel-blot hybridization of C15-1 with *Bam*HI-digested DNA from *Rht-B1a* (var. Mercia), *Rht-B1b* (Mercia near-isogenic line) and two *Rht-B1g* homozygotes. A hybridizing 2.5-kb *Bam*HI fragment (assigned to chromosome 4B by nullisomic-tetrasomic analysis; data not shown) is missing in the *Rht-B1g* samples.

© 1999 Macmillan Magazines Ltd

Figure 4 Basmati rice is dwarfed by a construct containing the *gai* ORF. **a**, Primary transformants were allowed to set seed (by self-pollination), and seedling gibberellin-response tests[12] were performed on the progeny. Results from two independent transformant families are shown. Each pair of seedlings consists of two seedlings from the same family: left, a segregant lacking the transgene and displaying the classical elongation response to applied gibberellin; right, a segregant that contains the transgene and is relatively unresponsive to the applied gibberellin. **b**, Adult plant phenotypes. Right, tall plant lacking the transgene is a segregant from the same family as the dwarf plant (left) that contains the transgene.

that we have cloned maize *d8*. The protein encoded by *d8* is closely related to that encoded by the wheat *GAI*-like genes (d8 shows 88% amino-acid identity with Rht-B1a and Rht-D1a), and these genes all map to the same region of the 'ancestral' cereal genome[17]. Thus it is reasonable to assume that maize *d8* and the wheat *GAI*-like genes are the same gene (are orthologues) in these two species. As a whole, our results show that each of five independent dominant mutant alleles (at *d8* and *Rht-1*) is associated with a mutation in this orthologous *GAI*-like gene, demonstrating that we have cloned *Rht-1* and *d8*. The deletion in *Rht-B1g* provides further confirmation that *Rht-B1* is an orthologue of *Arabidopsis GAI*, and that *Rht-B1b* is a mutant allele of this cloned gene.

Height reduction has been associated with yield increases and yield stability in a number of different crop species[3]. Dwarfing mutant alleles of *GAI*, *Rht-1* or *d8* can now be used directly to reduce the height of diverse crops. As a test of this, we introduced constructs expressing the gai protein into Basmati 370 rice (Fig. 4a, b). This rice is commonly grown in northern and north-western regions of the Indian subcontinent. Basmati 370 grain is popular because it is long and slender, is translucent white, cooks well and has a pleasant aroma. However, the plants are tall, with weak culms (stems), and are highly susceptible to damage by wind and rain. This damage causes considerable yield losses and a reduction in grain quality. Previous attempts (using conventional breeding methods) to reduce the height of Basmati 370 while retaining its good qualities were not successful owing to loss of the unique characters for which it is valued. In our experiments, seedling segregants carrying the gai-expressing construct exhibited reduced responses to gibberellin, whereas segregants lacking the transgene responded normally to gibberellin (Fig. 4a). Adult plants carrying the transgene were dwarfed with respect to control segregants lacking the transgene (Fig. 4b). It is now possible to insert a single, genetically dominant, potentially yield-enhancing, dwarfing gene into the genome of any transformable crop, without the need for long-term conventional breeding programmes and with minimal disruption of genetic background.

Our results show that *Arabidopsis GAI*, wheat *Rht-1* and maize *d8* are functional orthologues. Gibberellin signalling appears to be very similar in monocotyledonous and dicotyledonous plants, and may involve the interaction of an SH2-like domain with a phosphorylated tyrosine residue. The mutations in the dominant dwarfing alleles of *D8* and *Rht-1*, like the mutation in the *gai* allele, affect the N-terminal region of the proteins that they encode. Previously, we proposed that GAI is a growth repressor whose action is opposed by gibberellin, and that gai is a mutant repressor that is relatively insensitive to the effects gibberellin[6,9]. According to this view, our data show that a range of different N-terminal deletions and truncations convert GAI/Rht-B1a/Rht-D1a/d8 into mutant repressors that are less affected by gibberellin than the normal protein. This confirms the importance of this N-terminal region for gibberellin signalling and is also consistent with the 'altered function' mode of dominance exhibited by the dominant mutant alleles of *GAI*, *Rht-1* and *d8*[6,7,9,12]. Gibberellin elicits plant responses in a dose-dependent fashion[15]. The fact that different dominant mutant alleles of *Rht-1* and *d8* confer differing severities of dwarfism[4–7] indicates that one of the functions of GAI/RGA/Rht-B1a/Rht-D1a/d8 may be to modulate the gibberellin dose-response. Different amino-terminal deletions and truncations may differentially alter the magnitude of response to a given gibberellin dose, and the structure of this amino-terminal region may be key to the modulator function of GAI/RGA/Rht-B1a/Rht-D1a/d8. □

Methods

Molecular cloning, DNA gel-blot hybridization and DNA sequencing. We isolated wheat cDNA and genomic DNA and maize genomic DNA clones using low-stringency library screens[25]. Wheat DNA gel-blot hybridizations were performed as described[26]. Wheat genomic DNA clones were assigned to their chromosome of origin (4A, 4B or 4D) by identification of restriction fragments

© 1999 Macmillan Magazines Ltd

previously assigned through DNA gel-blot analysis of nullisomic-tetrasomic lines. DNA sequencing was done using the Big Dye terminator cycle sequencing kit (Perkin Elmer). The entire coding sequence of each mutant gene (and of wild-type controls) was amplified from genomic DNA (using primers specific to *Rht-B1*, *Rht-D1* or *d8*, as appropriate) by PCR (GeneAmp XL PCR kit, Perkin Elmer). All wild-type and mutant *Rht-1* alleles were amplified from homozygous material. Amplification products were cloned into the pGEM-T Easy vector (Promega). For each gene, we determined DNA sequences from at least two independent amplifications, thus avoiding potential PCR-induced errors. Genetic analyses confirmed that the mutant *D8-1* and *Rht-D1b* sequences co-segregated with their respective mutant phenotypes. For *D8-1*, PCR analysis of the F_1 progeny of a *D8-1/d8* × *d8/d8* cross revealed five dwarf (*D8-1/d8*) plants that were heterozygous for the deletion mutation associated with *D8-1* (see text) and five tall (*d8/d8*) plants that did not carry this deletion. For *Rht-D1b*, the associated nucleotide substitution (see text) was found in three dwarf (*Rht-D1b/Rht-D1b*) but not in three tall (*Rht-D1a/Rht-D1a*) F_2 progeny of a *Rht-D1a/Rht-D1a* × *Rht-D1b/Rht-D1b* cross.

Isolation of *Rht-B1g*. We irradiated 3,000 wheat seeds (var. Highbury, homozygous for *Rht-B1b*) with 3.0 Gy fast-neutrons. *Rht-B1g* was identified as a tall, gibberellin-responsive[12] segregant in an M_2 family derived from self-pollination of an M_1 plant.

Rice transformants. We generated transgenic rice plants expressing the *Arabidopsis* gai protein by particle-gun-mediated transformation[27] using a construct in which the *gai* ORF was expressed under the control of the maize ubiquitin promoter. Presence of the *gai*-containing transgene was verified by PCR amplification[9]. The progeny (derived from self-pollination) of six independent primary transgenic plants were tested for segregation of the transgene and for gibberellin response[12]. In all six families, the transgene and phenotype were perfectly co-segregated: all plants exhibiting a normal gibberellin response lacked a detectable transgene, and all plants exhibiting a reduced gibberellin response contained the transgene. Thus, the reduced gibberellin response phenotype is due to the transgene, and not to inactivation of genes resulting from insertion of the transgene into the rice genome, or to genetic variation generated by the transformation procedure *per se*. Control transformants containing the vector but lacking *gai* were not dwarfed (data not shown).

Illustrations. The amino-acid sequence alignments in Fig. 2a were done using software from the Wisconsin Package (Genetics Computer Group) with default parameters. Alignments in Fig. 2b were made by eye.

Received 8 March; accepted 17 May 1999.

1. Dyson, T. *Population and Food: Global Trends and Future Prospects* (Routledge, London, 1996).
2. Conway, G. *The Doubly Green Revolution: Food For All in the 21st Century* (Penguin Books, London, 1997).
3. Evans, L. T. *Crop Evolution, Adaptation and Yield* (Cambridge Univ. Press, Cambridge, 1993).
4. Gale, M. D. & Youssefian, S. in *Progress in Plant Breeding* (ed. Russell, G. E.) 1–35 (Butterworths, London, 1985).
5. Börner, A., Plaschke, J., Korzun, V. & Worland, A. J. The relationships between the dwarfing genes of wheat and rye. *Euphytica* **89,** 69–75 (1996).
6. Harberd, N. P. & Freeling, M. Genetics of dominant gibberellin-insensitive dwarfism in maize. *Genetics* **121,** 827–838 (1989).
7. Winkler, R. G. & Freeling, M. Physiological genetics of the dominant gibberellin non-responsive maize dwarfs, *Dwarf8* and *Dwarf9*. *Plant.* **193,** 341–348 (1994).
8. Koornneef, M. *et al.* A gibberellin insensitive mutant of *Arabidopsis thaliana*. *Physiol. Plant.* **65,** 33–39 (1985).
9. Peng, J. *et al.* The *Arabidopsis GAI* gene defines a signalling pathway that negatively regulates gibberellin responses. *Genes Dev.* **11,** 3194–3205 (1997).
10. Koch, C. A., Anderson, D., Moran, M. F., Ellis, C. E. & Pawson, T. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* **252,** 668–674 (1991).
11. Hooley, R. Gibberellins: Perception, transduction and responses. *Plant Mol. Biol.* **26,** 1529–1555 (1994).
12. Gale, M. D. & Marshall, G. A. The chromosomal location of *Gai1* and *Rht1* genes for gibberellin insensitivity and semi-dwarfism, in a derivative of Norin 10 wheat. *Heredity* **37,** 283–289 (1976).
13. Webb, S. E., Appleford, N. E. J., Gaskin, P. & Lenton, J. R. Gibberellins in internodes and ears of wheat containing different dwarfing alleles. *Phytochemistry* **47,** 671–677 (1998).
14. Fujioka, S. *et al.* The dominant non-gibberellin-responding dwarf mutant (*D8*) of maize accumulates native gibberellins. *Proc. Natl Acad. Sci. USA* **85,** 9031–9035 (1988).
15. Peng, J., Richards, D. E., Moritz, T., Caño-Delgado, A. & Harberd, N. P. Extragenic suppressors of the Arabidopsis *gai* mutation alter the dose-response relationship of diverse gibberellin responses. *Plant Physiol.* **119,** 1199–1208 (1999).
16. Silverstone, A. L., Ciampaglio, C. N. & Sun, T.-p. The *Arabidopsis RGA* gene encodes a transcriptional regulator repressing the gibberellin signal-transduction pathway. *Plant Cell* **10,** 155–169 (1998).
17. Moore, G., Devos, K. M., Wang, Z. & Gale, M. D. Grasses, line up and form a circle. *Curr. Biol.* **5,** 737–739 (1995).
18. Di Laurenzio, L. *et al.* The *SCARECROW* gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. *Cell* **86,** 423–433 (1996).
19. Schumacher, K., Schmitt, T., Rossberg, M., Schmitz, T. & Theres, K. The *Lateral Suppressor* (*Ls*) gene of tomato encodes a new member of the VHIID protein family. *Proc. Natl Acad. Sci. USA* **96,** 290–295 (1999).
20. Chen, X. *et al.* Crystal structure of a tyrosine phosphorylated STAT-1 dimer bound to DNA. *Cell* **93,** 827–839 (1998).
21. Becker, S., Groner, B. & Müller, C. W. Three-dimensional structure of the Stat3 homodimer bound to DNA. *Nature* **394,** 145–151 (1998).
22. Darnell, J. E. Jr STATs and gene regulation. *Science* **277,** 1630–1635 (1997).
23. Fütterer, J. & Hohn, T. Translation in plants—rules and exceptions. *Plant Mol. Biol.* **32,** 159–189 (1996).
24. Peng, J. & Harberd, N. P. Derivative alleles of the *Arabidopsis* gibberellin-insensitive (*gai*) mutation confer a wild-type phenotype. *Plant Cell* **5,** 351–360 (1993).
25. Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, 1989).
26. Devos, K. M., Dubcovsky, J., Dvorák, J., Chinoy, C. N. & Gale, M. D. Structural evolution of wheat chromosomes 4A, 5A, and 7B and its impact on recombination. *Theor. Appl. Genet.* **91,** 282–288 (1995).
27. Sudhakar, D. *et al.* An efficient rice transformation system utilizing mature seed-derived explants and a portable, inexpensive particle bombardment device. *Transgenic Res.* **7,** 289–294 (1998).
28. Kurata, N. *et al.* A 300-kilobase-interval genetic map of rice including 883 expressed sequences. *Nature Genet.* **8,** 365–372 (1994).
29. Winkler, R. G. & Helentjaris, T. Dominant dwarfs. *Maize Genet. Coop. Newsl.* **67,** 110–111 (1993).
30. Kawata, T. *et al.* SH2 signalling in a lower eukaryote: a STAT protein that regulates stalk cell differentiation in *Dictyostelium*. *Cell* **89,** 909–916 (1997).

Acknowledgements. We thank S. Cobb, E. Coen, G. Coupland and C. Dean for comments on the manuscript; L. Sayers for help with figures; A. Harvey, D. Lonsdale and T. Dyer for the wheat/maize cDNA/genomic DNA libraries; the International Atomic Energy Agency (Vienna) for mutagenesis; T. Helentjaris for additional mapping and DNA sequencing; T. Sasaki of the Japanese Rice Genome Programme for EST clones; and the BBSRC (Core Strategic Grant to the John Innes Centre; Plant Molecular Biology II; Agricultural Systems Directorate), EU Framework IV and Pioneer Hi-Bred International for funding.

Correspondence and requests for materials should be addressed to N.P.H. (e-mail: harberd@bbsrc.ac.uk).
EMBL data library accession numbers are: AJ242530, AJ242531.

Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1

Jon Chambers*, Robert S. Ames†, Derk Bergsma†, Alison Muir*, Laura R. Fitzgerald‡, Guillaume Hervieu§, George M. Dytko‡, James J. Foley‖, John Martin¶, Wu-Schyong Liu¶, Janet Park*, Catherine Ellis†, Subinay Ganguly#, Susan Konchar#, Jane Cluderay§, Ron Leslie§, Shelagh Wilson* & Henry M. Sarau‖

*Departments of *Molecular Screening Technologies and §Neuroscience, New Frontiers Science Park, SmithKline Beecham Pharmaceuticals, Southern Way, Harlow, Essex CM19 5AW, UK*
Departments of †Molecular Biology, ‡Renal Pharmacology, ‖Pulmonary Pharmacology, ¶Protein Biochemistry, #Gene Expression Sciences, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, Philadelphia 19406, USA

The underlying causes of obesity are poorly understood but probably involve complex interactions between many neurotransmitter and neuropeptide systems involved in the regulation of food intake and energy balance. Three pieces of evidence indicate that the neuropeptide melanin-concentrating hormone (MCH) is an important component of this system. First, MCH stimulates feeding when injected directly into rat brains[1,2]; second, the messenger RNA for the MCH precursor is upregulated in the hypothalamus of genetically obese mice and in fasted animals[1]; and third, mice lacking MCH eat less and are lean[3]. MCH antagonists might, therefore, provide a treatment for obesity. However, the development of such molecules has been hampered because the identity of the MCH receptor has been unknown until now. Here we show that the 353-amino-acid human orphan G-protein-coupled receptor SLC-1 (ref. 4) expressed in HEK293 cells binds MCH with sub-nanomolar affinity, and is stimulated by MCH to mobilize intracellular Ca^{2+} and reduce forskolin-elevated cyclic AMP levels. We also show that SLC-1 messenger RNA and protein is expressed in the ventromedial and dorsomedial nuclei of the hypothalamus, consistent with a role for SLC-1 in mediating the effects of MCH on feeding.

© 1999 Macmillan Magazines Ltd